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Molecular cloning and chromosomal localization of one of the human glutamate receptor genes

(kainate receptor/chromosomal region 5q33/α-amino-3-hydroxy-5-methyl-4-isoxasolepropionic acid)

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ABSTRACT **Glutamate receptors are the predominant excitatory neurotransmitter receptors in the mammalian brain and are classified on the basis of their activation by different agonists. The agonists kainate and α-amino-3-hydroxy-5-methyl-4-isoxasolepropionic acid define a class of glutamate receptors termed kainate receptors. We have isolated and sequenced a human glutamate receptor (GluHI) cDNA and determined the chromosomal localization of its gene. The DNA sequence of GluHI would encode a 907-amino acid protein that has a 97% identity to one of the rodent kainate receptor subunits. Many of the changes between the predicted amino acid sequence of GluHI and the most similar rodent kainate receptor (GluRI) occur in a region of the protein encoded in rodents by an alternatively spliced exon. The extreme conservation between the human and rat kainate receptor subunits suggests that a similar gene family will encode human kainate receptors. The GluHI mRNA is widely expressed in human brain. The human gene encoding the GluHI subunit is located at 5q33. While the GluHI gene is not located near a chromosomal region associated with any human neurogenetic disorders, the homologous region on mouse chromosome 11 contains the sites of five neurologic mutations.**

Glutamate receptors are the predominant excitatory neurotransmitter receptor in the mammalian brain and are activated in a variety of normal neurophysiological processes (1, 2). The classification of glutamate receptors has been based on their activation by different pharmacological agonists. Glutamate receptors have therefore been named according to their respective agonists, the *N*-methyl-D-aspartate (NMDA), quisqualic acid (QUIS), kainate (KA), and 2-amino-4-phosphonobutyrate (AP4) receptors (1). Quisqualate receptors are also activated by the more selective agonist α-amino-3-hydroxy-5-methyl-4-isoxasolepropionic acid (AMPA).

The molecular cloning of cDNA for a rodent glutamate receptor (GluRI) has led to significant advances in our understanding of KA- and AMPA-gated glutamate receptors (3). Four additional types of KA receptor subunit cDNAs have been isolated from rat with various degrees of nucleotide and amino acid sequence identity to GluRI (3–7). Comparison of these sequences suggests that glutamate receptors sensitive to KA and AMPA are encoded by a family of closely related genes. The coexpression of different complementary RNA (cRNA) transcripts derived from these cDNAs in *Xenopus* oocytes has shown that (*i*) the respective protein products can interact to form heteromeric receptors; (*ii*) the agonists KA and AMPA can activate the same receptor or receptor subunit combinations; and (*iii*) different combinations of receptor subunits display distinct electrical properties (3–7). The extended family of related KA/AMPA receptor genes in rats suggests that, if these genes are conserved, multiple receptor subunit genes will also be used to generate diversity in human KA receptors.

Human glutamate receptors are of medical interest because of their postulated role in the mediation of excitation-induced neuronal cell death, particularly in stroke and inherited neurodegenerative disorders (8, 9). To determine if glutamate receptors are involved in any inherited neurodegenerative disorders will require the molecular cloning of human glutamate receptor genes and the determination of any linkage of these genes to inherited neurodegenerative disorders. To begin this analysis, we have characterized the cDNA for a human glutamate receptor subunit gene¶ and determined its chromosomal localization.

MATERIALS AND METHODS

Isolation of Human cDNAs. Poly(A)$^+$ RNA was isolated from the brains of Wistar rats. One microgram of rat brain poly(A)$^+$ RNA was transcribed with avian myeloblastosis virus reverse transcriptase. A pair of 34-residue oligonucleotides with a 10-nucleotide (nt) *Xho* I site on a 5′ end were synthesized:

KA1 5′-GACTCGAGTCGACCGGGGCCTGTC-AGTCGTGCAG-3′

and

KA2 5′-GACTCGAGTCACAGTCCCCAGCATT-CCCCCGGCG-3′.

The last 24 nucleotides of KA1 correspond to nucleotides 400–424 and the last 24 nucleotides of KA2 correspond to the complement of nucleotides 894–918 of the published sequence of GluRI (5). The oligonucleotides KA1 and KA2 were used as primers in a polymerase chain reaction (PCR) with the rat brain cDNA. This PCR product was gel purified and random primed (10). About 400,000 recombinants of a human brainstem–cerebellum λGT11 cDNA library (11) were screened with this radiolabeled probe under reduced strin-

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Abbreviations: KA, kainate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxasolepropionic acid; cRNA, complementary RNA; nt, nucleotide.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M64752).

gency conditions [40% (vol/vol) formamide/5× SSPE at 37°C (1× SSPE is 180 mM NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA)]. Nylon filters were washed to a final stringency of 0.5× SSPE at 50°C.

Nucleotide Sequence Determination and Analysis of Predicted Amino Acid Sequence. Nucleotide sequence was determined by the use of the chain-termination method. Sequence analysis and comparisons were performed with the Wisconsin General Computing Sequence Analysis Package and version 66 of the GenBank and European Molecular Biology Laboratory data banks (12, 13). Amino acid alignments were performed with the CLUSTAL program, utilizing gaps of up to 10 amino acids and a gap penalty of 10 (14). A phylogenetic comparison was performed with the PAUP program (15).

Genomic Blotting. Genomic blots of human DNA cleaved with the restriction enzymes *Bam*HI, *Eco*RI, *Hin*dIII, and *Xba* I were prepared as described (16). A genomic blot utilizing field inversion electrophoresis on human genomic DNA cleaved with *Sfi* I and *Sal* I was generously provided by Kai Wang. All blots were hybridized at high stringency (50% formamide/4× SSPE at 42°C) and washed to a final stringency of 0.2× SSPE at 60°C.

RNA Blots and Ribonuclease Protection Assays. Northern blots were performed with 5 μg of poly(A)$^+$ RNA isolated from postmortem human brain and hybridized and washed under the conditions described above for genomic blots. Ribonuclease protection assays (17) were performed with 5 μg of total RNA, and protected fragments were electrophoresed on denaturing 5% polyacrylamide gels and examined by autoradiography. The antisense cRNA probe used to detect mRNA for the human glutamate receptor GluHI was derived from a 500-base-pair (bp) *Pst* I–*Eco*RI fragment from the 5′ end of GluHI cDNA cloned in pBluescript KS+ (Stratagene). The antisense cRNA control was derived from a 300-bp fragment of mouse β-actin. Both probes were hybridized in the same reaction and digested, and they gave protected fragments of 475 bp for the GluHI cRNA and multiple bands including the 220-bp one for the β-actin probe.

Isolation of Human Genomic Clones. Two genome equivalents of a human cosmid library were screened with random-primed probe at high stringency by colony hybridization.

Chromosomal Preparation. Prometaphase chromosomes were prepared from peripheral blood lymphocytes by using the bromodeoxyuridine synchronization method (18).

***In Situ* Hybridization.** Cosmid DNAs were labeled with biotin-11-dUTP (Sigma) by using nick translation. Preannealing the probe and *in situ* hybridization were performed as described by Lichter *et al.* (19) with some modification.

To generate clear reverse bands (20), chromosome preparations were then counterstained with chromomycin A3 followed by distamycin (J.K., unpublished method), a modification of the procedure of Magenis *et al.* (21). To simultaneously see both the fluorescein isothiocyanate (FITC) signal and the banding pattern, Zeiss filter set 05 was utilized and the resulting image was photographed with Kodak technical pan film.

RESULTS

Isolation and Characterization of the Human KA Receptor cDNA. To isolate a human glutamate receptor cDNA, we screened, under reduced stringency conditions, a human brain cDNA library with a rodent probe. The probe was derived by PCR amplification of reverse-transcribed rat brain RNA by using oligonucleotide primers based on the sequence of the rat KA/AMPA-sensitive receptor GluRI. This screen yielded eight positive λ clones. One clone, designated GluHI, contained a 3.1-kilobase (kb) insert, and the sequence of this clone was determined by chain termination methods on both strands. The predicted protein encoded by the GluHI cDNA is 907 amino acids (Fig. 1) with molecular weight of 100,000. The predicted amino acid sequence of GluHI has 97% identity with the rodent GluRI (3). There is a potential signal peptide after the first ATG of the longest open reading frame.

GluHI Identifies a Widely Expressed mRNA. KA receptors have been shown to be widely expressed in rodent brain (4). GluHI identified a single 5.5-kb message on RNA blots of poly(A)$^+$ RNA isolated from human brainstem and cerebellum (Fig. 2*A*). Ribonuclease protection assays on total RNA indicate that GluHI is widely expressed in human brain. The relative order of abundance of message identified by GluHI antisense cRNA is cerebellum > temporal cortex ≥ frontal cortex ≈ hypothalamus > spinal cord (Fig. 2*B*). The GluHI message is not identified in human muscle (Fig. 2*B*, lane 6).

GluHI Identifies a Single Gene. Genomic blots of human DNA probed with GluHI cDNA demonstrate a large number of fragments under high-stringency conditions (data not shown). These restriction endonuclease fragments minimally encompass 50–60 kb. When GluHI is used as a probe on human genomic DNA cleaved with *Sfi* I and *Sal* I, only a single *Sal* I band is identified: 250 kb (data not shown). These results suggest that the human KA receptor subunit GluHI is encoded by a gene between 50 and 250 kb in size. Since the GluHI cDNA is 2.5 kb smaller than the message identified in human brain RNA, it probably would not identify all of the genomic fragments encoding the 3′ region of the GluHI mRNA.

Genomic Cloning of Human KA Receptor Subunit. The human cDNA (GluHI) was utilized as a probe to screen a human cosmid library under stringent conditions. Three cosmids were isolated from this screen, designated KAG1.1, KAG2.1, and KAG3.1. Restriction digests of these cosmids demonstrated that they contained inserts between 32 and 35 kb in length. On the basis of the fragment sizes observed after cleavage with different restriction endonucleases, each cosmid appears to be an independent clone but to have overlapping fragments.

The Gene Encoding GluHI Is Located at 5q33. To determine the genomic locations of the three cosmids (KAG1.1, KAG2.1, and KAG3.1), fluorescence *in situ* hybridization was utilized. Each of the three cosmids independently identified a single site of hybridization on chromosome 5 band q33 in high-quality metaphase preparations. Clean signals were noted on both chromatids of both chromosomes 5 in 50% of metaphase cells, on three chromatids in 40%, and on two chromatids in 10% of cells. Over 300 cells were evaluated in five independent experiments using KAG3.1. No other sites of hybridization were noted on both chromatids of any other chromosome band (Fig. 3). The precise band location was identified by using chromosomes of about 650 bands and employing reverse banding with chromycin A3 and distamycin.

Experiments utilizing the simultaneous hybridization of KAG3.1 and KAG2.1 revealed occasional closely spaced double signals arranged diagonally on each of the single chromatids. Although this may indicate the presence of two closely spaced genes, it may also represent two independent signals emanating from the ends of a single gene or an artifact of unknown significance occasionally seen with single cosmid hybridization. These possibilities can be resolved by using two-color fluorescence hybridization of interphase material.

DISCUSSION

The degree of identity and similarity (allowing for conservative amino acid substitutions) between the human cDNA product and the five rat KA receptor clones and chicken and frog KA-binding proteins is represented in Fig. 4. Comparison of the nucleotide sequences of the five rodent cDNAs

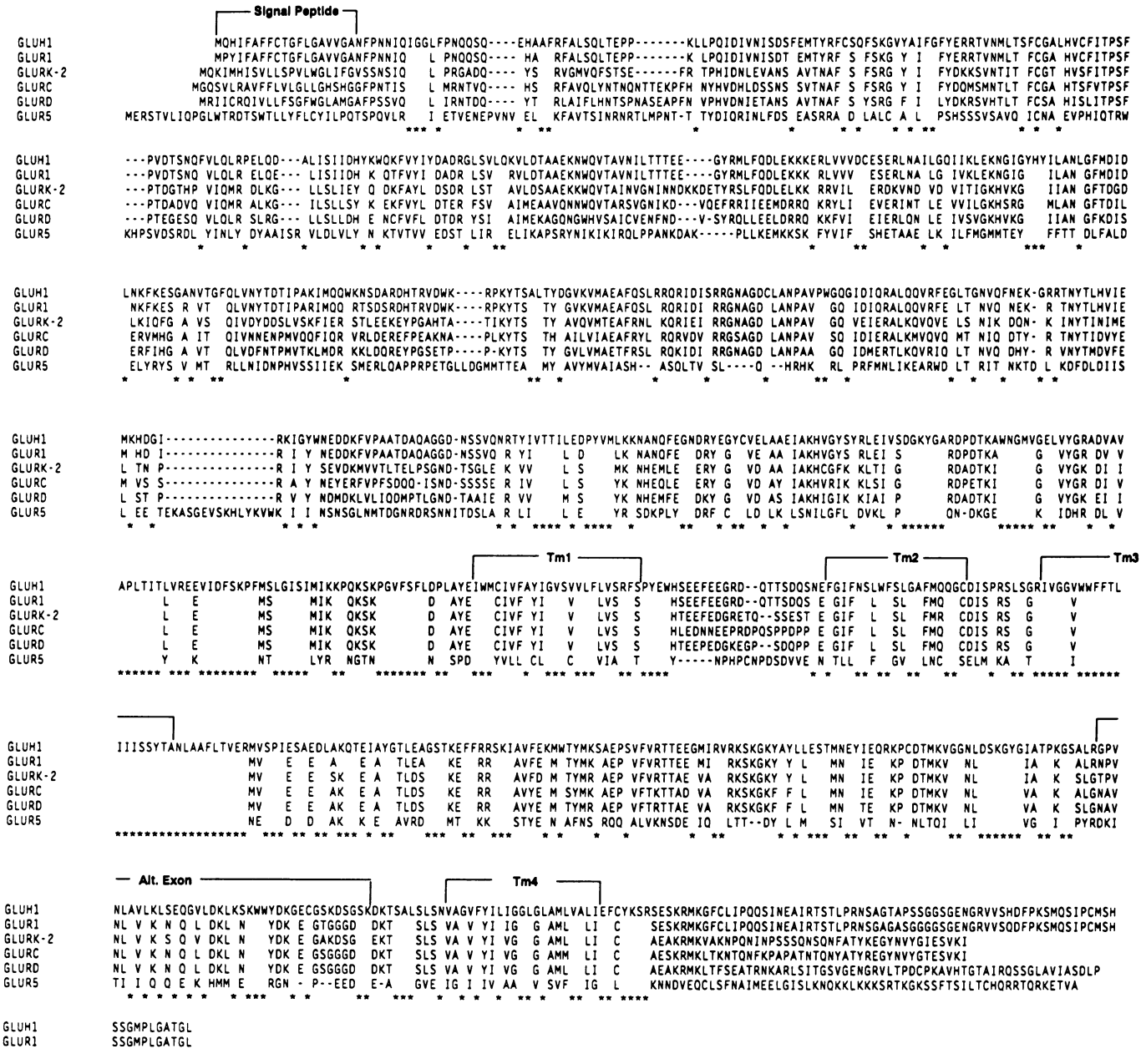

FIG. 1. Comparison of the amino acid sequence of the human KA receptor subunit cDNA GluHI with rodent KA receptor subunits. The predicted amino acid sequence of GluHI is shown on the top line. Below are the rat KA receptor subunits in order of their amino acid identity to GluHI. The alignment was performed by CLUSTAL analysis (14). The most amino-terminal and carboxyl-terminal regions of the predicted amino acid sequence were not aligned. Gaps are indicated by -. Identical amino acids shared by GluHI and the other KA receptors are indicated by open spaces and an asterisk below the sequences. Transmembrane regions proposed by Keinänen *et al.* (4) for the rodent KA receptors are indicated above the similar regions of GluHI by the designation Tm. The alternatively spliced exon (Alt. Exon) found in the "flip" and "flop" versions of rodent KA receptors is indicated above the similar sequence in GluHI (22). The deduced amino acid sequences are from GluRI (3), GluR-K2 (5), GluRC, GluRD (4), and GluR5 (7).

suggests that the respective mRNAs are unlikely to be generated by the alternative splicing of a single gene. A phylogenetic comparison (Fig. 4) of the human and rodent KA receptors and chicken (23) and frog (24) KA-binding proteins suggests that these genes arose from multiple duplication events that preceded the divergence of frogs and chickens from mammals. Extrapolating from the diversity of KA receptors seen in rodents, we expect that a similar diversity is likely to be found in human KA receptors. We also expect that this diversity will be generated by a similar family of genes.

The proposed structure of ligand-gated channels is based on four or more membrane-spanning α-helices and an extracellular amino-terminal region that would interact with the ligand (25). The locations of the proposed transmembrane (Tm) regions predicted by Keinänen *et al.* (4) for the rodent glutamate receptors are shown above the identical regions of GluHI in Fig. 1. These same regions are predicted to be membrane-spanning α-helices by Nakanishi *et al.* (5) in their analysis of KA receptor cDNAs. Within the GluHI sequence there are seven potential transmembrane regions 20 amino acids or greater in length predicted by the hydrophobic moment analysis of Eisenberg *et al.* (26). While the location of these membrane-spanning regions cannot be predicted with certainty (27), the evolutionary conservation in amino acid sequence in this region among different subunits and between species suggests a strong selection against nonsynonymous substitutions.

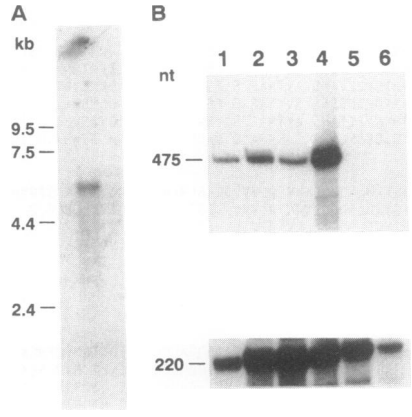

FIG. 2. Analysis by Northern blot (*A*) and ribonuclease protection assay (*B*) of the expression of GluHI mRNA. (*A*) Northern blot of 5 μg of poly(A)$^+$ RNA from human cerebellum probed with GluHI, demonstrating a single 5.5-kb message. (*B*) Ribonuclease protection assay of the relative level of expression of GluHI mRNA in various regions of the human central nervous system. The 475-nt band identifies the probe protected by GluHI mRNA, while the 220-nt band identifies the relative amount of β-actin mRNA protected by the mouse β-actin probe. Lane 1, frontal cortex; lane 2, temporal cortex; lane 3, hypothalamus; lane 4, cerebellum; lane 5, spinal cord; lane 6, muscle. Brain tissue was all from a single adult individual but represents various amounts of gray and white matter from a gross dissection. The amount of protected β-actin probe in lanes 1 and 6 is clearly lower in this experiment. When the assay was repeated and equal amounts of protected β-actin probe were seen in these lanes, the relative abundance of GluHI mRNA in different brain regions was similar.

Many of the amino acid differences (9 of 25 amino acids) between GluHI and GluRI are in a single 38-amino acid region defined by Sommer *et al.* (22). This region separates the third and fourth predicted membrane-spanning regions (Tm3 and Tm4) and is strongly conserved in KA receptors, but it shows considerable divergence in other ionotropic receptors. In rodent KA receptors this region is encoded by alternatively spliced exons. The amino acids encoded by this exon in rodent KA/AMPA receptors are indicated above the same region of GluHI in Fig. 1. The alternative splicing of the exons encoding this region produces KA-sensitive receptor subunits with different agonist and desensitization profiles. Sommer *et al.* (22) have designated these different types of receptor subunits "flip" and "flop." The human cDNA encoding GluHI would be considered as the flip counterpart to the flop version of the rodent clone GluRI (5). The conservation of the sequences encoding the flip type of receptor in GluHI suggests that the alternative splicing of similar exons will be used in human glutamate receptor genes.

FIG. 3. *In situ* localization of the GluHI gene to human chromosomal region 5q33. A human chromosome preparation hybridized with the cosmid KAG3.1 labeled with biotin-11-dUTP. The two arrows indicate hybridization to the q33 region of two distinct chromosomes 5.

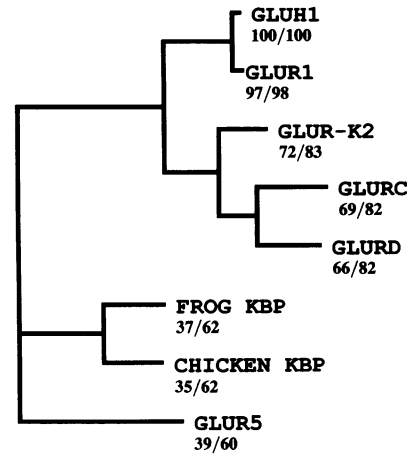

FIG. 4. Phylogram comparing the degree of relatedness in amino acid sequence between human GluHI, the rodent KA receptor subunits, and chicken and frog KA-binding proteins (KBP). The rodent sequences used for this comparison are those utilized and cited in Fig. 1. The other deduced amino acid sequences are from chicken KBP (23) and frog KBP (24). The degree of identity and similarity between GluHI and the other kainate cDNAs is indicated in numbers below representing percent identity/percent similarity as determined by a FASTA comparison (13).

The gene encoding this subunit of the KA receptors is localized to the q33 region of human chromosome 5 (Fig. 3). Two other receptor genes have been mapped to this band. They include the colony-stimulating factor 1 receptor (CSF1R) and platelet-derived growth factor β receptor (PDGFRB). These two receptors are within 0.5 kb of each other and are believed to have arisen by a duplication event, since they are significantly similar (28). The human γ-aminobutyric acid receptor A subunit ($GABA_A$-R) has also been mapped to 5q34–35 by *in situ* hybridization (29). Although these genes all encode cell surface receptors, they do not share any obvious similarity to GluHI at the amino acid level. Nevertheless, it is certainly possible that the PDGFRB, CSF1R, and GluHI genes are clustered in this region of human chromosome 5. However, the assignment of both CSF1R and PDGFRB to 5q33 is less precise than the present assignment because of the lower resolution of the techniques used to map them. Therefore, the GluHI gene may not be as close as currently indicated to the CSF1R and PDGFRB genes.

Although human chromosome 5 is composed of regions homologous to five mouse (*Mus musculus*) chromosomes, band 5q33 falls in a small region of overlap between homologous regions that are located on chromosomes 11 (MMU11) and 18 (MMU18) (30, 31). It is of interest that five neurogenetic mutations in the mouse have been mapped to a 5-centimorgan region located in the homologous region on MMU11 (32). These include shaker-2 (*sh-2*) (33), spasmodic (*spd*) (34), vibrator (*vb*) (35), tipsy (*ti*) (36), and trembler (*Tr*) (37). It would therefore be of interest to use the human cosmids KAG1.1, KAG2.1, and KAG3.1 to map the gene encoding the mouse counterpart of this subunit and determine its segregation with these mouse mutations. The *vb* mutant is especially interesting because the expression of this specific glutamate receptor subunit is highest in the cerebellum, one of the areas affected in the spinocerebellar degeneration seen

in *vb* mutants (35). Although the precise region of homology is less well mapped on MMUY18, we note a further five mutations on this chromosome that may be of interest when map positions of the homologous region are defined (32).

While it has not been shown that excitoxicity is involved in any human neurological disorder, this concept remains an attractive hypothesis in explaining disorders characterized by neuronal degeneration (9). However, there are no inherited human neurological disorders known to map near the GluHI gene at 5q33. The closest genetically determined neurological disorder in humans are two forms of spinal muscular atrophy that map to the 5q11–13 region (38). Linkage analysis has also shown an association between anonymous markers on 5q and schizophrenia in several Icelandic and British families (39). This association was not seen in other pedigrees (40). However, it would be interesting to see if the GluHI gene segregates with affected individuals in the British and Icelandic families. The molecular cloning of additional human glutamate receptor genes will be necessary to confirm the conservation of this gene family in humans. Determining the chromosomal location of each member of this gene family will establish whether the glutamate receptor genes map near any genes associated with neurogenetic disorders in mouse or man.

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1. Monaghan, P. T., Bridges, R. J. & Cotman, C. W. (1989) *Annu. Rev. Pharmacol. Toxicol.* **29,** 365–402.
2. Barnard, E. A. & Henley, J. M. (1990) *Trends Pharmacol. Sci.* **11,** 500–507.
3. Hollmann, M., O'Shea-Greenfield, A., Rogers, S. W. & Heinemann, S. (1989) *Nature (London)* **342,** 643–648.
4. Keinänen, K., Wisden, W., Sommer, B., Werner, P., Herb, A., Verdoorn, T. A., Sakmann, B. & Seeburg, P. H. (1990) *Science* **249,** 556–560.
5. Nakanishi, N., Schneider, N. A. & Axel, R. (1990) *Neuron* **5,** 569–581.
6. Boulter, J., Hollmann, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C. & Heinemann, S. (1990) *Science* **249,** 1033–1037.
7. Bettler, B., Boulter, J., Hermans-Borgmeyer, I., O'Shea-Greenfield, A., Deneris, E. S., Moll, C., Borgmeyer, V., Hollmann, M. & Heinemann, S. (1990) *Neuron* **5,** 583–595.
8. Olney, J. W., Ho, O. L. & Rhee, V. (1971) *Exp. Brain Res.* **14,** 61–70.
9. Choi, D. W. (1988) *Neuron* **1,** 623–624.
10. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
11. Kamholz, J., de Ferra, F., Puckett, C. & Lazzarini, R. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4962–4966.
12. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **72,** 387–395.
13. Pearson, W. R. & Lipman, D. J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2444–2448.
14. Higgins, D. G. & Sharp, P. M. (1988) *Gene* **73,** 237–241.
15. Swofford, D. L. (1990) PAUP, Phylogenetic Analysis Using Parsimony (Illinois Natural History Survey, Champaign, IL), Version 3.0.
16. Reed, K. C. & Mann, D. A. (1985) *Nucleic Acids Res.* **13,** 7207–7221.
17. Lee, J. J. & Costlow, N. A. (1987) in *Methods Enzymol.* **152,** 633–648.
18. Fan, Y.-A., Davis, L. M. & Shows, T. B. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6223–6227.
19. Lichter, P., Tang, C.-j. C., Call, K., Hermanson, G., Evans, G. A., Housman, D. & Ward, D. C. (1990) *Science* **247,** 64–69.
20. International Conventional System for Human Cytogenic Nomenclature (1985) in *Cytogenetics–Cell Genetics*, eds. Harnden, D. G. & Klinger, H. P. (March of Dimes Birth Defects Foundation, New York).
21. Magenis, R. E., Donlon, T. A. & Tomar, D. R. (1985) *Hum. Genet.* **69,** 300–303.
22. Sommer, B., Keinänen, K., Verdoorn, T. A., Wisden, W., Burnashev, N., Herb, A., Köhler, M., Takagi, T., Sakmann, B. & Seeburg, P. H. (1990) *Science* **249,** 1580–1585.
23. Gregor, P., Mano, I., Maoz, I., McKeown, M. & Teichberg, V. I. (1989) *Nature (London)* **342,** 689–692.
24. Wada, K., Dechesne, C., Shimasakai, S., King, R., Kusano, K., Buosnanno, A., Hampson, D., Banner, C., Wenthold, R. & Nakatani, Y. (1989) *Nature (London)* **342,** 684–689.
25. Unwin, N. (1989) *Neuron* **3,** 665–676.
26. Eisenberg, D., Schwartz, E., Komaromy, M. & Wall, R. (1984) *J. Mol. Biol.* **179,** 125–142.
27. Lodish, H. F. (1988) *Trends Biochem. Sci.* **13,** 332–334.
28. Roberts, M. W., Look, T. A., Roussel, M. F. & Sherr, C. J. (1988) *Cell* **55,** 655–661.
29. Buckle, V. J., Fujita, N., Ryder-Cook, A. S., Derry, J. M. J., Barnard, P. J., Lebo, R. V., Schofield, P. R., Seeburg, P. H., Buteson, A. N., Darlison, M. G. & Barnard, E. A. (1989) *Neuron* **3,** 647–654.
30. Lalley, P. A., Davisson, M. T., Graves, J. A. M., O'Brien, S. J., Womak, J. E., Roderick, T. H., Creau-Goldberg, N., Hillyard, A. L., Doolittle, D. P. & Rogers, J. A. (1989) *Cytogenet. Cell Genet.* **51,** 503–532.
31. Wasmuth, J. J., Park, C. & Ferrell, R. E. (1989) *Cytogenet. Cell Genet.* **51,** 137–138.
32. Doolittle, D. P., Hillyard, A. L., Guidi, J. N., Davisson, M. T. & Roderick, T. H. (1991) GBASE, the Genomic Database for the Mouse (The Jackson Laboratory, Bar Harbor, ME).
33. Deol, M. S. (1954) *J. Genet.* **52,** 502–588.
34. Lane, P. W., Ganser, A. L., Kerner, A. L. & White, W. F. (1987) *J. Hered.* **78,** 353–356.
35. Weimar, W. R., Lane, P. W. & Sidman, R. L. (1982) *Brain Res.* **251,** 357–364.
36. Searle, A. G. (1961) *Genet. Res.* **2,** 122–126.
37. Falconer, D. S. (1951) *J. Genet.* **50,** 192–201.
38. Gilliam, T. C., Brzustowicz, L. M., Castilla, L. H., Lehner, T., Penchaszadeh, G. K., Daniels, R. J., Byth, B. C., Knowles, J., Hislop, J. E., Shapira, Y., Dubowitz, V., Munsat, T. L., Ott, J. & Davies, K. E. (1990) *Nature (London)* **345,** 823–825.
39. Sherrington, R., Brynjolfsson, J., Petursson, H., Potter, M., Dudleston, K., Barraclough, B., Wusmuth, J., Dobbs, M. & Gurling, H. (1988) *Nature (London)* **336,** 164–167.
40. Kennedy, J. L., Giuffra, L. A., Moises, H. W., Cavalli-Sforza, L. L., Pakstis, A. J., Kidd, J. R., Castiglione, C. M., Sjogren, B., Wetterberg, L. & Kidd, K. K. (1988) *Nature (London)* **336,** 167–170.